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HLA Analysis of Immune Responders in Human Schistosomiasis

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Abstract

Background: Schistosomiasis is a serious global helminthic disease, in which the main immunopathology consists of a granulomatous and fibrosing reaction against tissue - trapped parasite eggs. In schistosomiasis *mansoni,* the pathogenesis of hepatosplenic disease has been shown to be due to immune mechanisms. The present study was designed to examine the relationship between the susceptibility versus resistance in schistosomiasis *mansoni* and the HLA antigens.

Material and methods: Two groups were examined: susceptible group to reinfection (83 patients) and resistant group (27 subjects) .

Results: The present results showed that the susceptibility was positively associated with the presence of HLA-B5 and negatively associated with HLA-B39 and HLA-DR14. These findings represent a step toward elucidating the factors controlling the pathogenic mechanisms in human schistosomiasis *mansoni.*

Key words : Human Leukocyte antigen (HLA) , Schistosomiasis , Immune Responders.

Introduction

Schistosomiasis, also known as bilharziasis, is estimated that 250 million people worldwide are infected with the snail-transmitted, water-borne parasitic helminth, and that 200,000 deaths every year are associated with the severe consequences of infection (WHO, 2008). Schistosomiasis mansoni is usually a chronic infection that leads to long-term systemic exposure to schistosome antigens, and that is associated with immunoregulatory mechanism (Watanabe *et al.,* 2007).

As long as a vaccine is not available, and the risk of infection in the areas where schistosome is endemic is not drastically reduced by a fundamental improvement of the socio-economical situation, the control of the disease is mainly achieved by chemotherapy of the patients. Despite effective chemotherapy, schistosomiasis remains a major public health problem in these developing countries. Rapid reinfection after treatment, accompanied by extensive residual morbidity, mandates alternative control strategies, including vaccine development (Jiz *et al.*, 2008).

According to Bergquist (1995), development of vaccine against this disease is now a WHO priority. More than six candidate vaccines against Schistosoma mansoni : paramyosin (Corrêa - Oliveira *et al.*,1989), glutathione S-transferase, GST (Boulanger *et al.*,1991), trios-phosphate isomerase, TPI (Harn *et al.*, 1992), Sm 23 (Koster *et al.*,1993), irradiation associated vaccine antigen IrV-5 (Soisson *et al.*,1993), glyceraldehydes -3- phosphate dehydrogenase (GPDH) (El Ridi *et al.*,1998, 2001 a,b), fatty acid binding protein, Sm14 (Cardoso *et al.*,2006).

Bergquist (1995), have been tested for reactivity with human cells and sera of donors with past or current S. mansoni infection. Currently, based on vaccine studies in vitro, schistosome vaccine became a realistic option. However, the mechanisms by which these work done, were still require further investigation, especially the role of human leukocyte antigen (HLA) and its association with immune responses, which could be correlated with resistance to reinfection.

Schwartz 1992, stated that HLA system is the most polymorphic genetic system known. The characteristic polymorphism of HLA molecules plays a critical

role in determining the immune response potential of the individual (IR gene effect) and consequent HLA and disease association.

The identification of Major histocompatiblity complex (MHC) has become a priority for the development of peptide-based prophylactic and therapeutic vaccines *(Depil et al., 2006)*. HLA studies in Egypt indicated that the host's genetics contribute to disease susceptibility (Blanton *et al.*, 2005). There is a strong association between the major histocompatiblity complex and schistosomiasis. (Reis *et al.*, 2008 a).

According to Lightowlers *et al.* (1993), variation in innate resistance to parasite infection is important in determining the prevalence and intensity of infection in the population. As such, the factors that influence innate resistance may play crucial roles in the success of parasite control and vaccination programs.

The study aims to characterize and identify the major subpopulations responsible for the induction of protective associated responses in human schistosomiasis and the role of HLA associated with immune responses.

Material And Methods

Donor Selection

The study participants were among a group of patients admitted to the Tropical Medicine Department of Theodore Bilharz Research Institute (TBRI). It included patients living in suburban areas surrounding Guiza Governorate (endemic area for schistosomiasis for many years). All investigations were done in accordance with the Ministry of Health and Human Service guidelines for clinical research and treatment under a protocol approved by the schistosomiasis research project, VACSERA, Egypt. All patients gave their informed consent before participation in this study.

Susceptible group

In the present study, 83 patients were observed. Individuals suffering from any parasitic infection other than *S. mansoni* were excluded from the study to eliminate possible interacting effects of any other co-existing parasitic infection, even the cases with mixed urinary and intestinal infection (Schinski *et al.*, 1976).

Resistant group

27 resistant persons were studied. It is very important to distinguish between resistance and lack of exposure as possible reasons for a lack of super infection or of reinfection after treatment (***Bradly et al., 1973***). The present study was carried on 110 patients (83 + 27) who were suffering initially (2002) from *S. mansoni* infection (detected by parasitological examination). All of them were treated by praziquantel (40 mg/kg B.W.) one to three doses 6 weeks apart until all of them were apparently cured (i.e., eggs were no longer detected on stool analysis).

In January 2003 the subjects (all of the 110), were re-examined parasitologically using stool analysis (Kato-Katz method) (Katz *et al.*, 1972). Active S. mansoni infection was detected by the number of eggs per gram stool (epg) (mean of two slides of each fecal sample was calculated). Stool analysis of 27 cases remained negative in spite of their frequent exposure to infected water either for swimming, washing or farming. And all of cases (110) were re-treated to make sure of status of resistance and susceptibility.

Follow up was continued, and in January 2004 all cases were re-examined parasitologically again in the same ways and the 27 cases remained negative under the same conditions. So this group was classified as the resistant group and the other 83 cases were classified as the susceptible group.

Blood Sampling

Peripheral blood was collected by vein puncture using heparinized venoject vacuum tube plasma was prepared by centrifugation of blood samples at 3000 rpm. Plasma was aliquated and stored at -20 °C for subsequent laboratory analysis.

HLA Typing

Biotest Lymphotype HLA kits were used for HLA typing as follow:

1. HLA-ABC 72 for HLA class I.
2. HLA-DR/DQ 72 for HLA class II.

- **Intended Use**

Biotest Lymphotype HLA is used for the detection of human HLA antigens in a

complement-dependent micro-lymphocytotoxicity test (Terasaki *et al.*, 1978; Mittal 1978; Danilovs *et al.*, 1980; Mueller-Eckhardt *et al.*, 1994; Bodmer *et al.*,1997) Biotest lymphotype HLA consists of ready-to use microplates containing pre-loaded anti-HLA reagents. The anti-HLA reagents can be monoclonal HLA antibodies or human polyclonal HLA antisera (Middleton *et al.*, 1992).

- **Principle of the Microlymphocytotoxicity Test**

For the determination of HLA antigens, HLA antibodies with known specificity were incubated with a lymphocyte suspension of the samples in the presence of complement. After the addition of lymphocytes to lymphotype the lymphocytes were lysed in the presence of the corresponding antibody and complement. This is made visible by using a stain (eosin). The assessment of lysed and non-lysed lymphocytes was carried out using an inverse phase contrast microscope.

Statistical analysis of the results

The data were analyzed with the aid of the program (SPSS) Statistical Package for Social Science Version 17.0 for windows.

To assess the statistical significance of association of different antigen frequencies (in different groups under study) with the disease susceptibility, Chi-squared test (χ^2) with Yates correction for continuity ***(John and Sons 1995)*** was applied .

Results

In this work three groups were studied:

1. Susceptible group, which included 83 cases.
2. Resistant group, which included 27 cases.

These groups were studied with the aims of demonstration of the potential immuneogenetic predis-position for susceptibility and resistance to *Schistosoma mansoni* with regard to HLA typing.

Statistical analysis of the distribution of HLA types in the study susceptible group as compared to the resistant group

All alleles from the two classes of HLA-genes in the two groups (resistant and susceptible) were collected to make a genetic pool which contained 329 alleles in R-group and 946 alleles in S-group.

Statistical analysis of HLA class I

HLA class I includes HLA-A, HLA-B and HLA-C

Statistical analysis of HLA-A

Table (1) and figure (1) showed 17 alleles of HLA-A. Comparison between resistant group (R) and susceptible group (S) showed no significant association between any of HLA-A alleles and schistosomiasis *mansoni.*

Statistical analysis of HLA-B

Table (2) and figure (2) showed 30 alleles of HLA-B. Comparison between resistant group (R) and susceptible group (S) showed a statistically significant positive association of ***HLA-B5*** with *S. mansoni* with relative risk = 4.074, also there was a statistically significant negative association in ***HLA-B39*** with relative risk = 0.256.

The same table showed two subclasses from B-alleles, Bw4 and Bw6 and their statistical analysis revealed no significant association between both of them and schistosomiasis *mansoni.*

Statistical analysis of HLA-C

Table (3) and figure (3) show 8 alleles of HLA-C. Comparison between resistant group (R) and susceptible group (S) showed no significant association between any of HLA-C alleles and schistosomiasis *mansoni.*

Statistical analysis of HLA class II

HLA class II includes HLA-DR and HLA-DQ.

Statistical analysis of HLA-DR

Table (4) and figure (4) show .22 alleles of HLA-DR. Comparison between resistant group (R) and susceptible group (S) showed a statistically significant negative association in ***HLA-DR14*** with relative risk =0.206.

Statistical analysis of HLA-DQ

Comparison between resistant group (R) and susceptible group (S) showed no significant association between any of ***HLA-DQ*** alleles and schistosomiasis *mansoni* as shown in table (5) and figure (5).

Table (1): Comparison between resistant (R) and susceptible (S) readings of the distribution (frequency and percentage) of different class I HLA-A types (antigens).

HLA-A Antigens	Frequency		Percentage %		P. value	RR
	R-Group	S-Group	R-Group	S-Group		
A1	11	29	3.3%	3.1%	0.80	
A2	11	24	3.3%	2.5%	0.44	
A3	2	8	0.6 %	0.8 %	1.00	
A9	1	0	0.3 %	0 %	0.25	
A10	2	5	0.6 %	0.5 %	1.00	
A11	2	10	0.6 %	1.1 %	0.74	
A23	2	10	0.6 %	1.1 %	0.74	
A24	2	9	0.6 %	1 %	0.73	
A26	0	4	0 %	0.4 %	0.57	
A28	3	13	0.9 %	1.4 %	0.77	
A29	3	6	0.9 %	0.6 %	0.70	
A30	8	13	2.4 %	1.4 %	0.19	
A31	2	0	0.6 %	0 %	0.06	
A32	1	2	0.3 %	0.2 %	1.00	
A33	1	13	0.3 %	1.4 %	0.13	
A36	2	3	0.6 %	0.3	0.60	
A69	0	2	0 %	0.2 %	1.00	

P > 0.05 = not significant. N. of alleles in R-group =329
P < 0.05 = significant (*). N. of alleles in S-group =946
P < 0.01 = highly significant (**).
R.R. = relative risk.
Blank space=does not apply (RR was calculated for significant relations only).

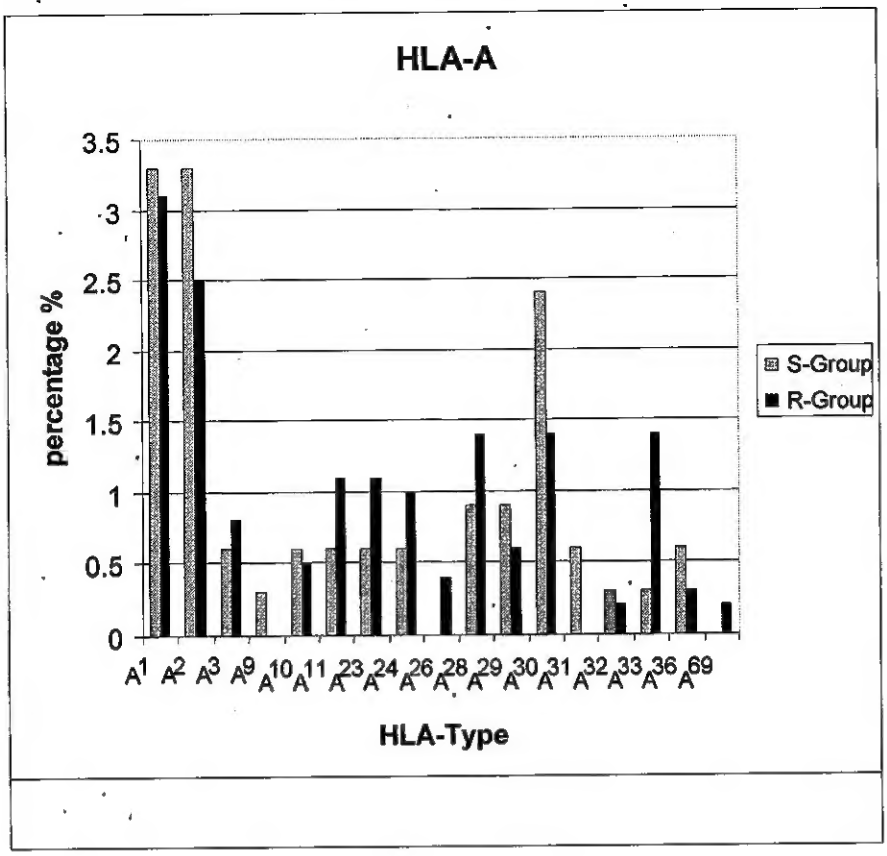

Fig. (1): Distribution of HLA-A antigens in susceptible group (S) as compared to the resistant group (R).

Table (2): Comparison between resistant (R) and susceptible (S) readings of the distribution (frequency and percentage) of different class I HLA-B types (antigens).

HLA-B Antigens	COUNT		PERCENTAGE %		P value	RR
	R-Group	S-Group	R-Group	S-Group		
B4	0	1	0 %	0.1 %	1.00	
B5	2	23	0.6%	2.4%	0.04*	4.074
B7	1	0	0.3 %	0 %	0.25	
B8	0	2	0 %	0.2 %	1.00	
B13	0	3	0 %	0.3 %	0.57	
B14	3	7	0.9 %	0.7%	0.72	
B15	0	2	0 %	0.2 %	1.00	
B18	1	8	0.3 %	0.8 %	o.46	
B22	0	2	0 %	0.2 %	1.00	
B27	1	5	0.3 %	0.5 %	1.00	
B31	2	0	0.6 %	0 %	0.60	
B35	8	16	2.4 %	1.7 %	0.39	
B38	3	8	0.9 %	0.8 %	1.00	
B39	4	0	1.2 %	0 %	0.004**	0.256
B41	4	12	1.2 %	1.3 %	1.00	
B44	1	10	0.3 %	1.1 %	0.30	
B45	0	2	0 %	0.2 %	1.00	
B47	0	1	0 %	0.1 %	1.00	
B49	0	3	0 %	0.3 %	0.57	
B50	3	9	0.9 %	1 %	1.00	
B51	4	3	1.2%	0.3 %	0.30	
B52	2	0	% 0.6	0 %	0.66	
B53	1	9	0.3 %	1 %	0.46	
B55	1	3	0.3 %	0.3 %	1.00	
B56	0	1	0 %	0.1 %	1.00	
B57	4	5	1.2 %	0.5 %	0.24	
B58	1	4	0.3 %	% 0.4	1.00	
B63	1	6	0.3 %	% 0.6	0.68	
B70	1	3	0.3 %	0.3 %	1.00	
B71	1	1	0.3 %	0.1 %	0.45	
Bw4	16	52	4.9%	5.5%	0.66	
Bw6	24	57	7.3%	6.0%	0.41	

P >0.05 = not significant.
P <0.05 = significant (*).
P <0.01 = highly s. (**).
R.R. = relative risk.

No. of alleles in R-group=329
No. of alleles in S-group=946

Blank space=does not apply (RR was calculated for significant relations only).

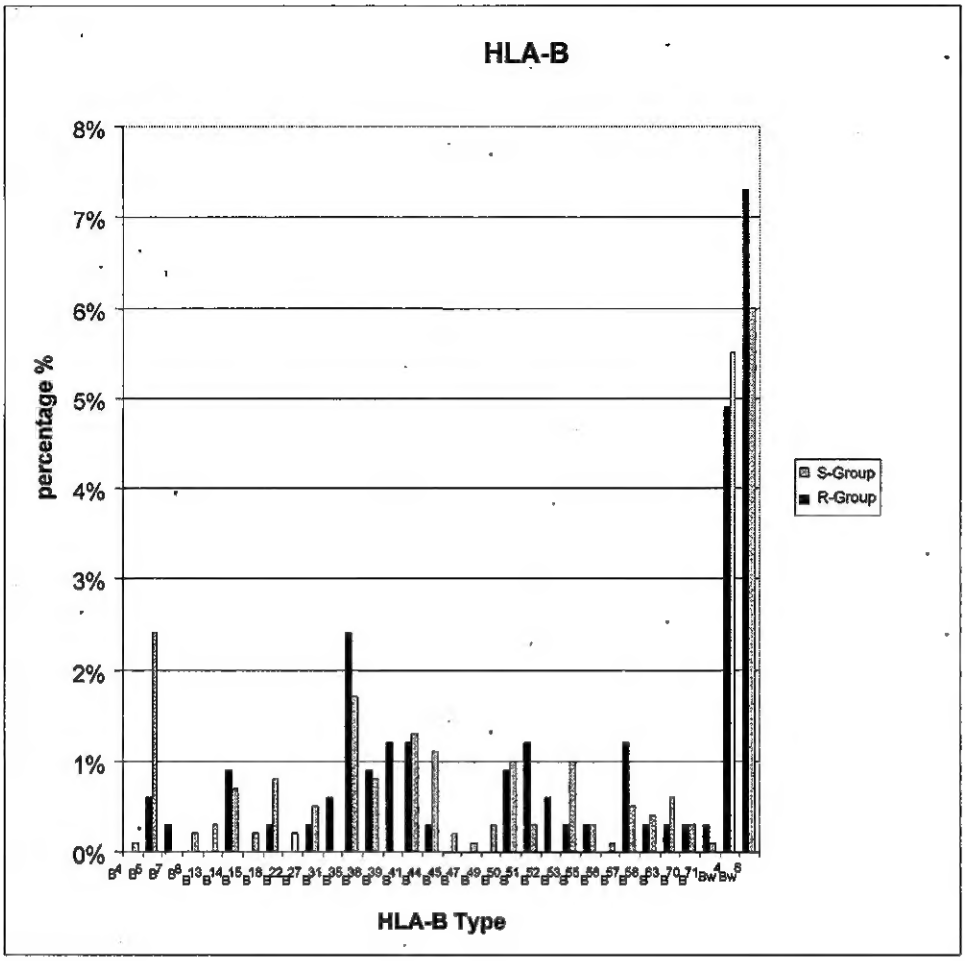

Fig. (2): Distribution of HLA-B antigens in susceptible group (S) as compared to the resistant group (R)

able (3): Comparison between resistant (R) and susceptible (S) readings of the distribution (frequency and percentage) of different class I HLA-C types (antigens).

HLA-C Antigens	FREQUENCY		PERCENTAGE %		P value	R.R.
	R-Group	S-Group	R-Group	S-Group		
CW2	0	6	0%	0.6%	0.34	
CW3	2	6	0.6%	0.6%	1.00	
CW4	13	37	4.0%	3.9%	0.97	
CW5	1	6	0.3%	0.6%	0.68	
CW6	18	37	5.5%	3.9%	0.23	
CW7	6	28	1.8%	3.0%	0.27	
CW8	5	8	1.5%	0.8%	0.33	
CW17	5	9	1.5%	1%	0.37	

P > 0.05 = not significant. N. of alleles in R-group = 329
P < 0.05 = significant (*). N. of alleles in S-group=946
P < 0.01 = highly significant (**).
R.R. = relative risk.
Blank space=does not apply (RR was calculated for significant relations only).

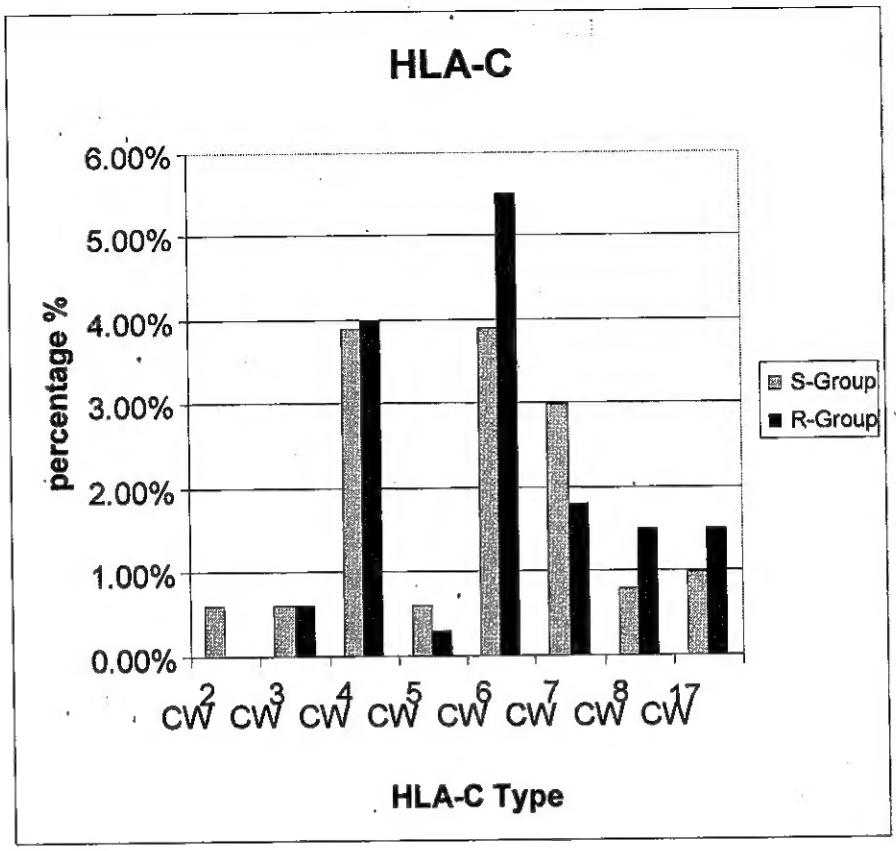

Fig. (3): Distribution of HLA-C antigens in susceptible group (S) as compared to the resistant group (R).

Table (4): Comparison between resistant (R) and susceptible (S) readings of the distribution (frequency and percentage) of different class II HLA-DR types (antigens).

HLA-DR Antigens	FREQUENCY		PERCENTAGE %		P value	R.R.
	R-Group	S-Group	R-Group	S-Group		
DR1	3	8	0.9%	0.8%	1.00	
DR2	1	0	0.3%	0%	0.25	
DR3	0	3	0%	0.3%	0.57	
DR4	11	32	3.3%	3.4%	0.97	
DR5	0	1	0%	0.1%	1.00	
DR7	3	17	0.9%	1.8%	0.26	
DR8	3	6	0.9%	0.6%	0.70	
DR9	2	0	0.6%	0%	0.06	
DR10	1	2	0.3%	0.2%	1.00	
DR11	5	23	1.5%	2.4%	0.33	
DR12	1	5	0.3%	0.5%	1.00	
DR13	5	24	1.5%	2.5%	0.28	
DR14	5	3	1.5%	0.3%	0.03*	0.206
DR15	7	15	2.1%	1.6%	0.51	
DR16	1	1	0.3%	0.1%	0.45	
DR17	3	11	0.9%	1.2%	1.00	
DR18	1	3	0.3%	0.3%	1.00	
DR31	0	1	0%	0.1%	1.00	
DR35	0	2	0%	0.2%	1.00	
DR51	10	14	3.0%	1.5%	0.07	
DR52	17	54	5.2%	5.7%	0.71	
DR53	13	46	4.0%	4.9%	0.49	

P >0.05 = not significant. N. of alleles in R-group=329
P <0.05 = significant (*). N. of alleles in S-group=946
P <0.01 = highly significant (**).
R.R. = relative risk.
Blank space=does not apply (RR was calculated for significant relations only).

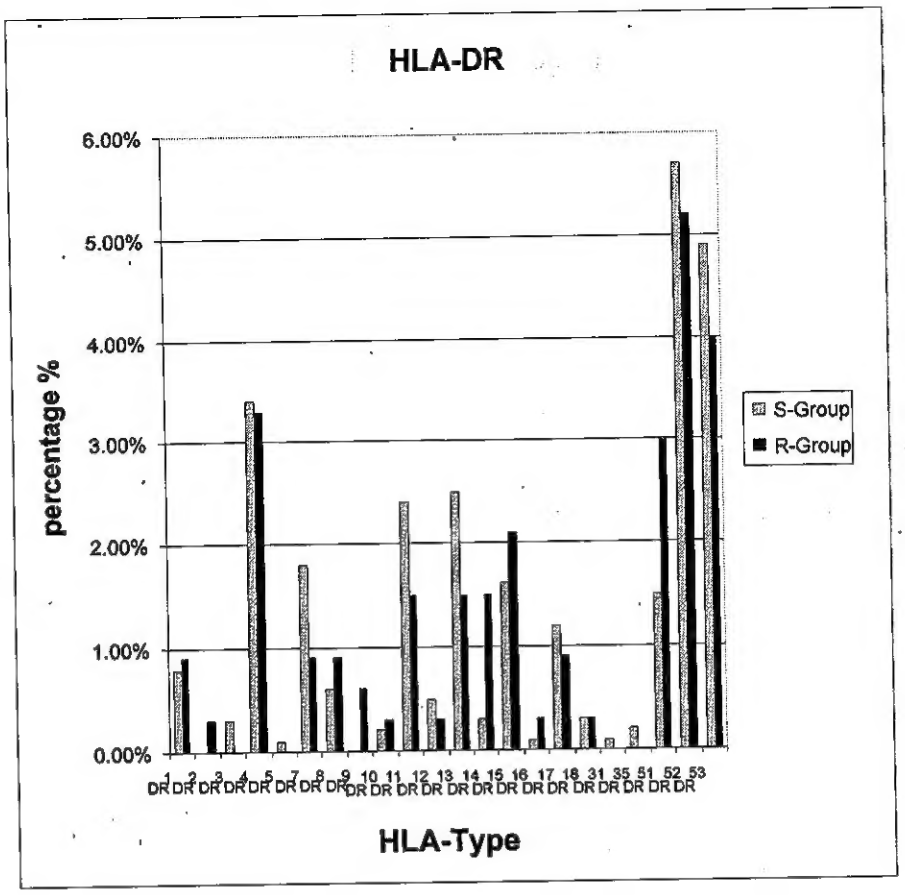

Fig. (4): Distribution of HLA-DR antigens in susceptible group (S) as compared to the resistant group (R).

Table (5): Comparison between resistant (R) and susceptible (S) readings of the distribution (frequency and percentage) of different class II HLA-DQ types (antigens).

HLA-DQ Antigens	FREQUANCY		Percentage %		P value	R.R.
	R-Group	S-Group	R-Group	S-Group		
DQ2	9	31	2.7%	3.3%	0.62	
DQ4	1	5	0.3%	0.5%	1.00	
DQ5	7	10	2.1%	1.1%	0.16	
DQ6	15	34	4.6%	3.6%	0.43	
DQ7	8	28	2.4%	3.0%	0.61	
DQ8	5	19	1.5%	2.0%	0.57	
DQ9	0	1	.0%	0.1%	1.00	

P > 0.05 = not significant.
P < 0.05 = significant (*).
P < 0.01 = highly significant (**).
R.R. = relative risk.
Blank space=does not apply (RR was calculated for significant relations only).

N. of alleles in R-group=329
N. of alleles in S-group=946

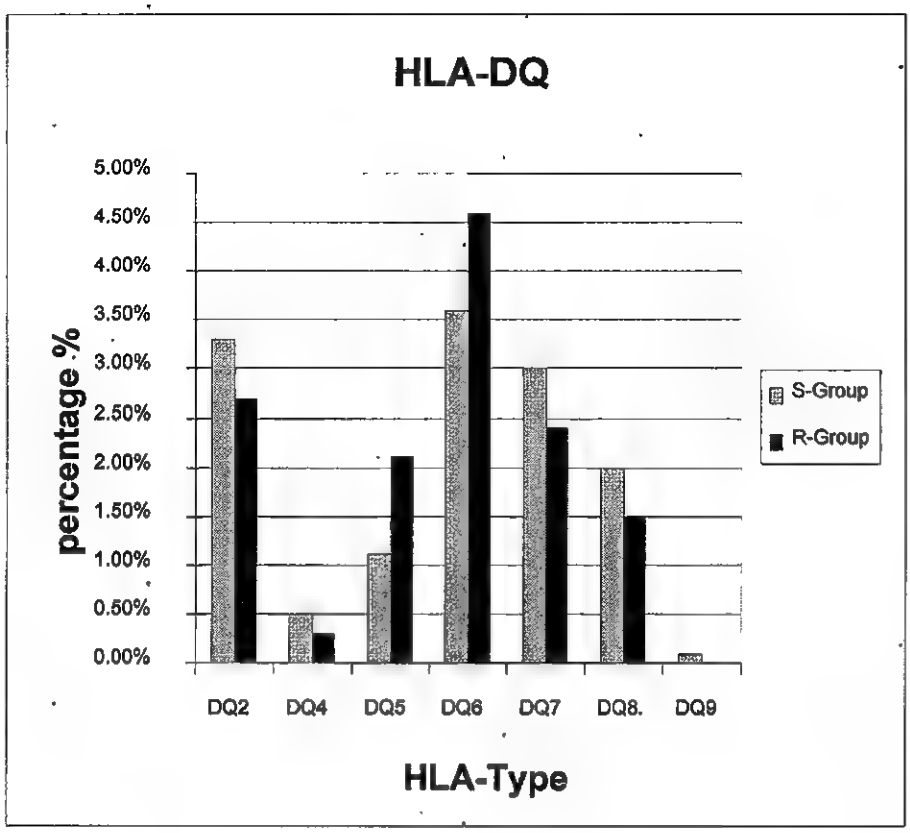

Fig. (5): Distribution of HLA-DQ antigens in susceptible group (S) as compared to the resistant group (R).

Discussion

Schistosoma mansoni is a major health problem for a number of developing countries (WHO, . 2008). It is found throughout most of sub-Saharan Africa, Egypt and Sudan, parts of the Middle East, northeastern parts of South America (including Brazil and the Guyanas), and the Caribbean. Its intermediate hosts are aquatic snails in the genus Biomphalaria. Reservoir host for S. mansoni include baboons and monkeys in Africa. However, they play no significant role in the epidemiology of human disease (Fuller *et al.*,1979). Infection with Schistosoma mansoni induces a wide range of effects on the immune responses of the host (Campi-Azevedo *et al.*, 2007).

Warren (1982 b) reported that, eradication of this parasite by vector control and chemotherapy has been attempted with some successes. The scaling up of these programs, however, is faced with numerous difficulties, and an efficient vaccine would represent a major step towards the control of this parasitic disease.

Several associations between various pathologies and specific HLA antigens have been reported (Tanija and David ,1999). In schistosomiasis, it is difficult to estimate the development of acquired resistance to reinfection, which is dependent on many factors such as daily exposure to the parasite (Remoue *et al.*, 2000). Recently, a genomic region involved in resistance has been described (Marquet *et al.*, 1996 &1999).

Most of the epidemiological studies focused on the factors involved in progression of fibrosis and development of sever hepatic disease. Lethal disease is a

consequence of portal hypertension, which progressively leads to hematemesis and finally to heart failure. In its early stage, fibrosis is part of the healing process that follows the acute inflammatory reaction around parasite eggs trapped in presinusoidal venules. Chronic hepatosplenomegaly is a consequence of extended fibrosis in the hepatoportal spaces. Sever hepatoportal disease was noted in certain families, while others living in the same environmental and hygienic conditions were less affected (Dessein *et al.*, 1999). Abdel-Salam *et al.* (1979) was the first to describe a linkage between progression toward hepatosplenomegaly and HLA class I antigens.

The present study consolidates the view of the important role of host immune reactivity in schistosomiasis mansoni and demonstrates the contribution of the genetic impact on immunological heterogeneity of the disease. These findings might support the genetic control of the disease or the presence of an immune response and/or immune suppression genes which are in linkage disequilibrium with these HLA antigens where they control the susceptibility and pathological sequences of the disease.

According to urine and stool examinations, all patients included in this study were free from other parasitic infections. Thus the results of HLA typing were not affected by any cross-reactions or immunological effects of other parasitic infections. HLA typing was done by using microcytotoxicity test, aiming to demonstrate the potential immunogenetic predisposition for susceptibility and resistance to S. mansoni.

In HLA class I, HLA-A showed no significant association between any of HLA-A alleles and schistosomiasis mansoni. While HLA-B revealed a statistically significant positive association of HLA-B5 with S. mansoni, also there was a statistically significant negative association in HLA-B39. HLA-C. Also no significant association between any of HLA-C alleles and schistosomiasis mansoni were found.

In HLA class II, HLA-DR a statistically significant negative association in HLA-DR14. While in HLA-DQ no significant association between any of HLA-DQ alleles and schistosomiasis mansoni was predicted .

Our results are in agreement with previous observations by several authors who have reported similar work in schistosomiasis and also contradicted with others. Abdel-Salam *et al.* (1979) found a positive association of schistosomal hepatosplenomegaly and two HLA antigens namely: HLA-A1 and HLA-B5 antigen. In 1982, Eissa demonstrated a significantly increased frequency of HLA-A26, A30, B5, B18 and a decreased frequency of HLA-B8 in patients suffering from hepatic schistosomiasis.

Also in 1986, Abdel-Salam *et al.* reported a positive association between HLA-B5 and B8 with schistosomal colonic polyposis. Another work of schistosomal colonic polyposis was done by Kamel et al. (1987) who reported the association of HLA-B5 and DR3 with the same disease. On the other hand, Zakaria *et al.* (1988) described a significant positive association of HLA-B5 and DR3 with schistosomal hepatosplenomegaly and negative association of DR2. El-Hawy *et al.* (1989) reported that HLA-B8 was positively associated with bilharzial hepatosplenomegaly.

Wishahi et al. (1989) reported that HLA-B7 was significantly increased in Egyptian patients with simple bilharzial cystitis and HLA-B16. While, Hafez et al., (1991) reported that the genetic susceptibility to hepatic fibrosis in Egyptian children was associated with a high frequency of HLA-A2 and B12 and a lack of DR2 antigens. On the other hand, Assaad-Khalil *et al.* (1993) found a significant association of HLA-DQ1 with failure to develop hepatosplenic disease. And Abdel-Fattah (1998) demonstrated a significant association of HLA-DR11 and DQ2 alleles with the schistosomiasis in Egypt.

As shown in the previous registrations the whole set of alleles was varied between different reports, but there was semi-consensus on HLA-B5 and its positive association with schistosomiasis which is in agreement with our results. It is obvious that B-5 has become a secret motif in the status of susceptibility and a strong reason to make the subject (who carries this allele) susceptible to reinfection with

schistosomiasis mansoni. As regards to B39, the present results may indicate an association between resistance and this allele, so, the subject with B39 may be named resistant to reinfection and he can face the infection challenge without fear of suffering from schistosomiasis later.

In HLA class II, also our results may indicate an association between HLA-DR14 and resistance status, so, the subject with HLA-DR14 may face the infection challenge safely.

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تقييم المولد المضاد لخلايا الدم البيضاء فى الإنسان للمستجابين مناعيا فى مرض البلهارسيا

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تمثل الإصابة بطفيل البلهارسيا أحد أهم الأمراض التى تؤثر سلبيا على الصحة العامة والاقتصاد القومى.

ولقد أظهرت الدراسات السابقة تذبذبا واضحا فى مستوى انتشار البلهارسيا بين المناطق المختلفة التى يستوطنها المرضى بمعنى أنه يوجد أفراد بعينهم مقاومين لتكرار العدوى بعد تناول العلاج للمرة الأولى بالرغم من استمرار تعرضهم للمياه الملوثة مما رجح وجود عامل وراثى خاص بمقاومة المرض يمكن هؤلاء الأفراد من بناء مناعة مكتسبة بعد الشفاء من أول إصابة مما يمنع تكرار العدوى مرة أخرى . وقد رجح أن هذا العامل الوراثى هو نوع المولد المضاد لخلايا الدم البيضاء.

اشتملت هذه الدراسة على110حالات تم تقسيمهم إلى مجموعتين تبعا لحالة الحساسية لتكرار العدوى (83حالة) فى مقابل مقاومة تكرار العدوى (27حالة) .

تم تحديد نوع المولد المضاد لخلايا الدم البيضاء لجميع الحالات فى محاولة للتوصل للبدائل المسئولة عن اكتساب الأفراد لحالة المقاومة وقد تم التوصل إلى أن المولد المضاد بى –5 يرتبط ارتباطا إيجابيا مع الحساسية لتكرار العدوى (الأشخاص الحاملين له ذوى حساسية لتكرار العدوى بنسبة خطأ لا تتجاوز 0.04٪) فى حين أوضحت النتائج الارتباط السلبى لكل من المولد المضاد بى – 39 و دى أر – 14 مع الحساسية لتكرار العدوى (الأشخاص الحاملين لها مقاومين لتكرار العدوى بنسبة خطأ لا تتجاوز 0.004٪ و 0.04٪ على الترتيب).